# Taguchi Approach to Process Design Optimization for Quality Improvement Effect of Parameters on Milk Kefir Fermentation

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## Abstract

Robust deign of experiment (DOE) has been widely utilized in many research areas. While there are several design methods, Taguchi orthogonal array method is faster and better mode of optimization providing remarkable effect in simultaneous study of several factors, producing good understanding with fewer experimental results and enhanced process performance showing high yield and validation. The aim of this work is to utilize the Taguchi approach in process design optimization of milk kefir fermentation parameters involving types of culture and fermentation time for enhanced kefir production from cow milk. The data collected from previous experimental work were subjected into Taguchi $L_9$ orthogonal array at appropriate statistical software and analyzed using 'smaller is best' criterion to determine the significance of each parameter. The pH of kefir was chosen as performance parameter, since it is substantial characteristic of milk kefir. The result indicated that fermentation time was significant factor on pH of milk kefir followed by types of culture.

**Keywords:** Design of experiment (DOE), Optimization, Taguchi method, Milk kefir

## Introduction

Several decades ago, the optimization of process parameters was performed by classical method. The method comprises varying one parameter at a time and keeping the other constant. But it is incompetent as it fails to understand relationships between the variables (reaction time, temperature, and substrate concentration) and response (percentage conversion). These procedures are time consuming, burdensome, require a lot of experimental data sets and do not exert information about the mutual interactions of the parameters. Statistical experimental design methods have been widely employed in bioprocess optimization, because these methods carefully explored the experimental space while studying various variables using a small number of observations (Adnani et al, 2010 optimization of lipase). On the other hand, technique of defining and investigation all possible conditions in an experiment involving multiple factors, known as the design of experiments (DOE) has been used in many research areas (Roy, 2010). Therefore, many control factors can be simultaneously studied and optimized.

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The Taguchi method has been widely used in manufacturing industry for decades to optimize the design of a product or process with a single quality characteristic (QC). Numerous successful applications of Taguchi methods have been studied to optimize several processes and improve product reliability and quality (Sasmal, 2011 Areca nut). The major principle of the Taguchi method is to improve the quality of a product by reducing the effect of the causes of variation without removing the causes. Three main requisites used in the Taguchi method are the orthogonal arrays, ANOVA (analysis of variance) and the signal to noise ratio (S/N). ANOVA is a matrix of numbers arranged in rows and columns. Each row represents the level of factors in each run and each column represents a specific level for a factor that can be changed for each run. S/N is an indicative of quality, and the purpose of the Taguchi experiment is to find the best level for each operating parameter to maximize S/N. Analysis of the experimental data using the ANOVA and effects of the factors gives the output that is statistically significant for finding the optimum levels (Adnani et al, 2010).

Taguchi method, as another way for designing factorial experiments, has some advantages, such as reducing time and cost by only a few experimental trials, providing a systematic and efficient plan for performing experiments under the consideration of the interactive effects among the control factors. This technique is somehow not very well known for optimization of biotechnological processes. The basic principle of this method is to screen filters examining the effects of many process variables and identify those factors having major effects on process using a few experiments (Dasu et al., 2003). Taguchi method of orthogonal array (OA) experimental design (DOE) includes a set of independent variables (factors) over a specific region of interest (levels). It focuses on the average process performance characteristics and considers the effect of variation on the process characteristics and makes the products or process performance insensitive to variation by proper design of parameters (Adnani et al, 2010; Prasad et al, 2005). In the method, main parameters which are assumed to have influence on process results are located at different rows in a designed orthogonal array. This approach helps to determine the influence of individual factors, providing the best performance at the optimum levels obtained by a few well–defined experimental sets (Adnani et al, 2010; Prasad et al, 2005).

The traditional method of handling the results of multiple samples per unit trial condition is to use the mean of the trial results to calculate the factor effects. A better way to compare the population behaviour is to use the mean square deviation (MSD) of the results. For convenience of linearity, and to accommodate wide range of data the log transformation of MSD, called signal to noise ratio (S/N) is recommended for analysis of the results. The term signal represents desirable value (mean) and noise represents the undesirable value (standard deviation from mean). Three types of S/N ratio based on calculation of MSD are robustly used to indicate the quality of optimisation process (Sasmal, 2011; Radhakumari et al, 2014).

The present work aimed to optimize the process parameters of milk kefir fermentation involving types of culture and fermentation time as control parameters subjecting into $L_9$ orthogonal array (OA) of Taguchi design.

## Methodology

### Taguchi Method

The Taguchi method is a robust design of experiments method based on OAs. It contributes a set of minimum number of experiments providing the complete information about the influence of all the factors on the performance parameter. The steps involved in Taguchi DOE methodology for optimization is shown in Fig 1.

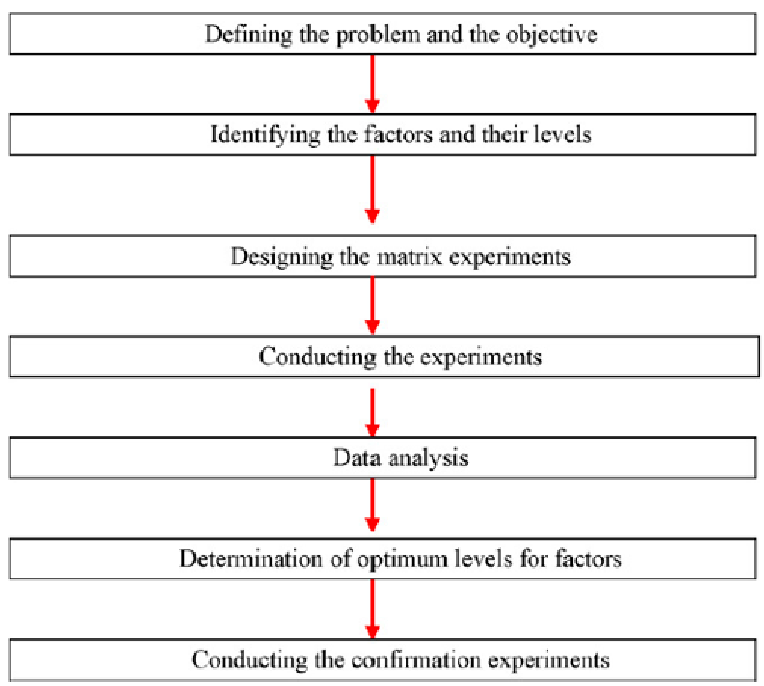


Fig 1. Flow chart of steps involved in Taguchi DOE methodology for optimization (Adnani et al, 2010)

### Taguchi Orthogonal Array Design

The first step of Taguchi procedure is to define the problem and objective. In this work, the problem is that Taguchi approach has not been fully utilized in milk kefir fermentation. Therefore, the aim of this work was to determine the optimal pH of milk kefir using Taguchi approach. After setting the objective, the next step is to assign the factors and their levels that influence the performance characteristics. Chosen factors should be independent variables that have significant effect on the performance. In this study, pH was chosen as performance parameter, since it was significant characteristic of milk kefir. Two factors were considered including types of culture (kefir grain, starter culture, mixed cultures) and fermentation time (12–36 h). Assigned factors and their corresponding levels are depicted in Table (1). $L_9$ ($3^4$) design was selected for experimental matrix, meaning it had totally 9 experimental runs with two columns representing assigned levels of each factor. The experimental design matrix is listed in Table (2).

After designing the experimental matrix, all of experiments were carried out according to previous work (May Thu Khaing, 2019). Data were collected and presented in Table (2).

Table (1) Assigned factors and their corresponding levels

| Symbol | Factors | Levels | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| A | Types of culture* | Kefir grain | Starter culture | Mixed culture |
| B | Fermentation time (h) | 12 | 24 | 36 |

* 2% w/v of culture was used for all experimental runs.

## Statistical analysis of Taguchi design

Observed data were analyzed in Minitab17® statistical software. The main function for optimization was provided by signal–to–noise ratio (S/N), a logarithmic function of the output preferred. The S/N ratio analogous to smaller–the–better objective function for the system was calculated using Eq.1 as follows (Radhakumari et al, 2014):

$$\frac{S'}{N} = -10\log_{10}\left[\frac{1}{n_j}\sum_{j=1}^{n} Y_i^2\right] \tag{1}$$

where, i is experiment number, n is number of replicates of experiment i, j is number of replicates and Y is performance parameter.

To determine the main effects of each factor involved in Taguchi experimental design, the response performance as pH and S/N ratio of all experiment runs were analyzed. The statistical significances of factors were determined by analysis of variance

(ANOVA) method. The optimal conditions were determined by combining the levels of factors having the highest main effects. 95% confidence interval, F ratio and the *p*–value ($p$ <0.05) were considered.

## Results and Discussion

Taguchi approach provides many standard orthogonal arrays and corresponding S/N ratio to determine the optimal conditions of best performance. OA design was used with two factor–three level, including types of culture and fermentation time. The observed data are given in Table 2. The objective function for optimization was used as smaller–the–better.

Table (2) $L_9$ orthogonal array of Taguchi method with observed data

| No. | Types of culture (A) | Fermentation time (B) | pH (response) |
|---|---|---|---|
| 1 | 1 | 1 | 5.0 |
| 2 | 1 | 2 | 4.6 |
| 3 | 1 | 3 | 4.3 |
| 4 | 2 | 1 | 5.1 |
| 5 | 2 | 2 | 4.5 |
| 6 | 2 | 3 | 4.3 |
| 7 | 3 | 1 | 5.2 |
| 8 | 3 | 2 | 4.6 |
| 9 | 3 | 3 | 4.0 |

### Statistical analysis – Effect of factors

The value of pH is substantial characteristic for milk kefir. Therefore, it was considered as performance parameter. In this regard, types of culture and fermentation time were assigned as independent factors that affected on pH of milk kefir. For all the factors, S/N ratio at each level was calculated based on observed data as shown in Table (2) using Eq. (1) and was listed in Table (3).

The delta range was calculated as difference of S/N ratios between maximum and minimum level of factor. Since the target of fermentation is to lower pH level of milk kefir, smaller–the–better criterion was considered to determine the optimal conditions. The maximum S/N ratio of each factor is desirable to obtain the lower pH level of milk kefir. The higher the delta range in each factor, the higher is the effect on pH of milk kefir. From

Table (3), it was notably observed that delta range value of fermentation time was higher than that of types of culture which is categorical factor.

Table (3) Response for signal to noise ratio (S/N) corresponding to pH

| Level | Types of culture (A) | Fermentation time (B) |
|---|---|---|
| 1 | –13.30 | –14.15 |
| 2 | –13.30 | –13.19 |
| 3 | –13.21 | –12.46 |
| Delta | 0.10 | 1.69 |
| Rank | 2 | 1 |

In addition, its value increased from level 1 to 3. Therefore, it was concluded that fermentation time had greater effect on pH parameter than the culture. It might be due to the fact that prolonged time increased the population of culture to produce more acid. However, effect of the use of kefir grain or start culture had no difference to change pH of milk kefir. Main effects of S/N ratios were plotted in Fig 2. From Fig 2, it was clearly seen that fermentation time was the most influent factor for milk kefir fermentation. This result was in agreement with previous literature (Magra et al, 2012).

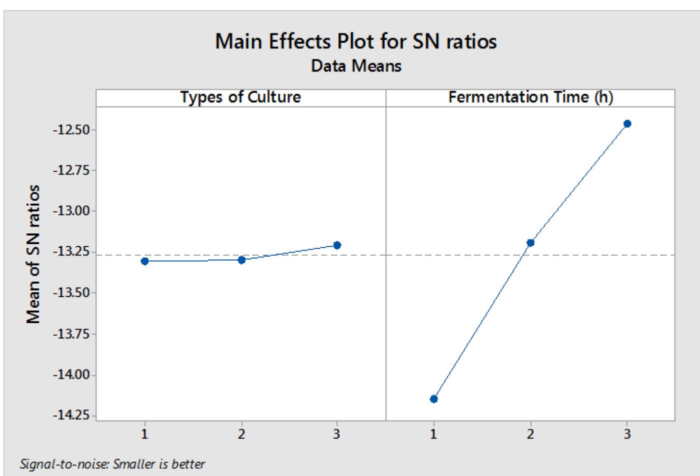


Fig 2. Main effects plot for S/N ratios

Table (4) presents analysis of variance (ANOVA). It confirmed that fermentation time had F–value of 36.14 indicating that it was statistically significant. Since 95% confidence interval and $p$<0.005 were selected to indicate the significant factor, fermentation time was significant factor implying that it had large effect on lowering pH value of milk kefir.

Table (4) Analysis of variance (ANOVA)

| Source | DF | Adj SS | Adj MS | F–value | *p*–value |
|---|---|---|---|---|---|
| Types of culture (A) | 1 | 0.00167 | 0.001667 | 0.10 | 0.767 |
| Fermentation time (B) | 2 | 1.22889 | 0.6144 | 36.14 | 0.001 |
| Error | 5 | 0.08500 | 0.170 | | |
| Total | 6 | 1.31556 | | | |

## Conclusion

Taguchi method can indicate the optimum combination of parameters to obtain desired pH value of milk kefir. The lowest pH was obtained with mixed cultures at 24 h of fermentation. Increase in fermentation time using mixed culture resulted in decreased pH level in milk kefir product. Taguchi approach can provide the faster and better mode of process optimization of milk kefir fermentation process reducing experiment time and cost.


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